

## **Certificate of Analysis for NR-537**

## Keystone Virus, B64-5587.05

Catalog No. NR-537

(Derived from ATCC® VR-722™)

**Product Description:** Cell lysate and supernatant from African green monkey kidney (Vero) cells<sup>1</sup> infected with Keystone virus, B64-5587.05.

Lot<sup>2</sup>: 4008228 Manufacturing Date: 20MAY2005

| TEST  | SPECIFICATIONS  | RESULTS   |
|---|---|---|
| Identification by Infectivity in Vero Cells <sup>1</sup>  | Cell rounding and detachment  | Cell rounding and detachment  |
| Titer by TCID <sub>50</sub> Assay <sup>3,4</sup> in Vero Cells <sup>1</sup>   | Report results  | 8.9 X 10 <sup>7</sup> TCID <sub>50</sub> /mL                                    |
| Sterility (21-day incubation)  Harpo's HTYE broth <sup>5</sup> , 37°C and 26°C, aerobic Trypticase soy broth, 37°C and 26°C, aerobic Sabouraud broth, 37°C and 26°C, aerobic Sheep blood agar, 37°C, aerobic Sheep blood agar, 37°C, anaerobic Thioglycollate broth, 37°C, anaerobic DMEM with 10% FBS, 37°C and 5% CO <sub>2</sub> | No growth | No growth |
| Mycoplasma Contamination  Agar and broth culture (14 day incubation at 37°C)  DNA Detection by PCR of extracted Test Article nucleic acid   | None detected<br>None detected  | None detected<br>None detected  |

<sup>&</sup>lt;sup>1</sup>Vero cells: ATCC<sup>®</sup> CCL-81™.

**Date:** 29 SEP 2006 **Signature:** Signature on File

**Title:** Technical Manager, BEI Authentication

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

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<sup>&</sup>lt;sup>2</sup>Grown in Minimum Essential Medium containing Earle's salts and non-essential amino acids (GIBCO<sup>®</sup> 10370-021) supplemented with 2% irradiated fetal bovine serum (Cambrex<sup>®</sup> 14-471E), 2 mM L-glutamine (GIBCO<sup>®</sup> 25030-081), and 1 mM sodium pyruvate (GIBCO<sup>®</sup> 11360-070) for 7 days at 37°C with 5% CO<sub>2</sub>.

<sup>&</sup>lt;sup>3</sup>The Tissue Culture Infectious Dose 50% (TCID₅₀) endpoint is the 50% infectious endpoint in cell culture. The TCID₅₀ is the dilution of virus that under the conditions of the assay can be expected to infect 50% of the culture vessels inoculated, just as a Lethal Dose 50% (LD₅₀) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the TCID₅₀ provides a measure of the titer (or infectivity) of a virus preparation.

<sup>&</sup>lt;sup>4</sup>7 days at 37°C and 5% CO<sub>2</sub>.

<sup>&</sup>lt;sup>5</sup>Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.